Cell, Vol. 97, 153–155, April 16, 1999, Copyright ©1999 by Cell Press

Brefeldin A: The Advantage of Being Uncompetitive

Minireview

Pierre Chardin and Frank McCormick
UCSF Cancer Center
San Francisco, California 94115

Brefeldin A is a small hydrophobic compound produced by toxic fungi that has dramatic effects on mammalian cells. Within minutes of brefeldin A treatment the Golgi complex disassembles and redistributes into the endoplasmic reticulum (ER): this makes it a potent inhibitor of secretion, and a very useful tool for cell biologists. What is the molecular basis of these remarkable effects? Brefeldin A inhibits some of the proteins that activate ADP-ribosylation factors (Arfs; Donaldson et al., 1992; Helms and Rothman, 1992). Small G proteins of the Arf family, in their active GTP-bound form, recruit and assemble protein complexes (the "coats") that participate in the selection of the desired set of cargo molecules and act as scaffolds to drive the budding of small membrane vesicles from donor membranes (reviewed by Schekman and Orci, 1996; see also Springer et al., 1999 and Roth, 1999, this issue of *Cell*). Vesicles budding from the ER fuse to form the intermediate compartment where a very active sorting takes place. Proteins that need to be secreted will progress by cisternal maturation and/or anterograde vesicular transport through the Golgi, whereas proteins that need to be retrieved to the ER will be sorted into retrograde vesicles for recycling (reviewed by Glick and Malhotra, 1998). Formation of these vesicles depends on the assembly of the COPI coat by Arf1-GTP. Inhibition of Arf1 leads to the release of the COPI coat and many other peripheral membrane proteins into the cytosol and ultimately results in the collapse of the Golgi and its redistribution into the ER (Dascher and Balch, 1994; Sciaky et al., 1997).

Yeast cells are usually impermeable, and thus resistant, to brefeldin A, but in permeable, mutant strains, brefeldin A blocks secretion and has dramatic effects on Golgi morphology, as it does in mammalian cells. In yeast, two proteins, Gea1 and Gea2, play an essential role in the early steps of Golgi trafficking. These proteins contain a region of approximately 200 amino acids that is similar to a domain of Sec7, a protein required for ER through Golgi transport in yeast. This "Sec7 domain" displays guanine nucleotide exchange activity for Arf1 (Chardin et al., 1996). Cathy Jackson's group has now shown that in yeast, the effects of brefeldin A on the secretory pathway are mainly due to the inhibition of the Gea1, Gea2, and Sec7 proteins (Peyroche et al., 1999).

Mammalian cells contain at least three classes of Arf exchange factors with high, moderate, or low sensitivity to brefeldin A. The collapse of the Golgi complex caused by brefeldin A is most likely due to the inhibition of Arf exchange factors; however, it is not clear which ones are the most important targets that explain the morphological effects of brefeldin A in mammalian cells. This aspect will be discussed briefly here; in this minireview, we would like to propose that the molecular mechanism of brefeldin A action might have important implications for drug discovery.

By what mechanism does brefeldin A inhibit Arf1 exchange factors? The characterization of the mutations present in *GEA1* alleles resistant to brefeldin A shows that the altered positions are clustered on a region of the Sec7 domain overlapping with, or very close to, the Arf1-binding site (Peyroche et al., 1999). Therefore, the most likely molecular explanation would be that brefeldin A competes with Arf for binding to the Sec7 guanine nucleotide exchange domain. If this were the case, it would be a rare example of a small molecule successfully blocking a protein:protein interaction. For this reason, the mechanism of brefeldin A action has been of great interest in the context of drug discovery as well as basic biology. However, when Anne Peyroche and Bruno Antonny tested this mechanism, they discovered that, unexpectedly, brefeldin A stabilizes an Arf:Sec7 domain complex rather than preventing its formation. This prompted detailed biochemical studies that have now shown that brefeldin A is an uncompetitive inhibitor: it binds to the transient complex formed between Arf-GDP and the Sec7 domain, leading to an abortive Arf-GDP:brefeldin A:Sec7 domain complex. In other words, brefeldin A makes endogenous Arf1-GDP behave as a "dominant negative" that traps the exchange factor and blocks activation of other Arf1 molecules by this particular exchange factor (Figure 1).

How Exchange Factors and the "Dominant-Negative" Mutants of Small G Proteins Work

The guanine nucleotide exchange factors (GEFs) for small G proteins first recognize the GDP-bound protein and lower the affinity for GDP, leading to a nucleotide-free transition state. GTP then enters the empty guanine-binding site and induces a conformational change that displaces the GEF (see Figure 7 of Peyroche et al., 1999). For Ras, and probably for most other G proteins, this last step is rate limiting for the exchange reaction and the release of Ras-GTP (Lenzen et al., 1998). The structure of the nucleotide-free form of Ras in complex with the Sos RasGEF domain has been solved, providing important insights on the details of these three steps (Boriack-Sjodin et al., 1998). Extensive use has been made of mutant forms of Ras where serine 17 is replaced by asparagine (RasN17); the corresponding mutations in related G proteins (Rac, Rho, etc.) have also been widely used to dissect signal transduction pathways. These mutant proteins act as dominant negatives, blocking activation of endogenous Ras proteins by upstream activators. In the RasN17 mutant, the affinity for GTP is decreased by three orders of magnitude compared to wild-type Ras; thus, GTP is no longer able to bind and displace the GEF, resulting in an abortive complex between nucleotide-free RasN17 and the GEF. Because exchange factors are usually expressed at lower levels than their target G proteins, low levels of RasN17 proteins are sufficient to sequester all exchange factors into abortive complexes, preventing endogenous Ras activation.

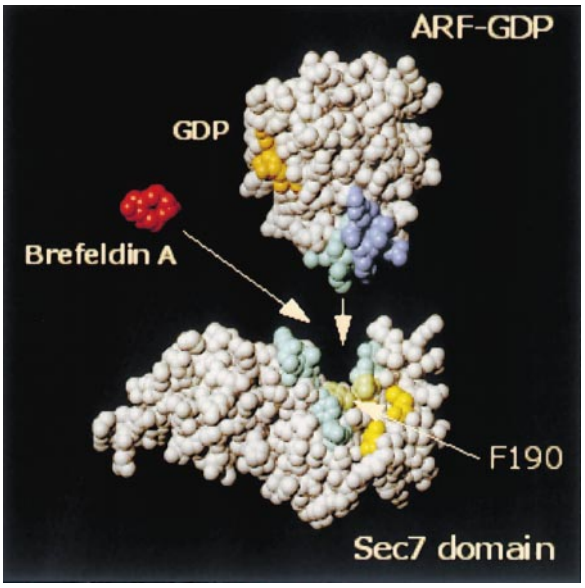

Figure 1. Proposed Mechanism of Brefeldin A Inhibition

(Top) Arf with GDP bound (orange), switch 1 region (light blue), and switch 2 region (dark blue) are the major sites of interaction with the Sec7 domain (Béraud-Dufour et al., 1998; Goldberg, 1998). (Bottom) Sec7 domain, major residues implicated in Arf1 interaction are in light blue, residues implicated in brefeldin A sensitivity in yellow, residues implicated in both are green. Phenylalanine 190 (corresponding to a tyrosine in Gea), one of the key residues that determine sensitivity, is indicated. Brefeldin A (modeled in red on the left) binds to the Arf-GDP/Sec7 domain complex, probably close to the yellow/green region of the Sec7 domain (helices H and I in the structure).

Different Arf Exchange Factors Have Different Sensitivities to Brefeldin A

There are at least four distinct families of Arf exchange factors, activating Arf1 or other members of the Arf family at different steps of the secretory and endocytic pathways. All of them share a common Sec7 domain that is responsible for the exchange activity and whose structure has recently been solved (Cherfils et al., 1998; Mossessova et al., 1998). A docking model for Arf-GDP on the Sec7 domain has also been proposed (Béraud-Dufour et al., 1998) and the structure of a nucleotide-free Arf:Sec7 domain complex has been solved (Goldberg, 1998). However, the motifs flanking this Sec7 exchange domain are different in each family, suggesting different modes of regulation. The "small" exchange factors (47 kDa) of the Arno/Cytohesin/GRP1 family contain an N-terminal coiled coil, a central Sec7 domain with a low sensitivity to brefeldin A, and a C-terminal PH domain that binds PIP3 (or PIP2). The PH domain is responsible for recruiting Arno to the membrane in response to signals that increase PIP3 (or PIP2) levels, possibly in a late stage of regulated secretion (Venkateswarlu et al., 1998). Proteins in two other families of exchange factors are much larger: bovine p200 is more closely related to yeast Sec7 and is highly sensitive to brefeldin A (Morinaga et al., 1997), whereas GBF1 is more closely related to yeast Gea1 and Gea2 (Mansour et al., 1998) and confers resistance to brefeldin A when overexpressed in mammalian cells (Yan et al., 1994). GBF1 and p200 are thought to function in ER-through-Golgi transport, as their yeast counterparts, but their regulation is poorly understood. Finally, there is the EFA-6 family of exchange factors that work preferentially on Arf6, the most distantly related member of the Arf family, and play an important role in early steps of endocytosis, and/or regulated exocytosis and actin cytoskeleton rearrangements at the plasma membrane (Franco et al., 1999). Mammalian cells of different origin have very different sensitivities to brefeldin A. For instance, in epithelial MDCK cells, the Golgi complex is surprisingly resistant to brefeldin A, but the AP-1/clathrin coat–dependent formation of vesicles on the *trans*-Golgi network, en route to the basolateral cell surface, is affected by brefeldin A (Orzech et al., 1999), suggesting that Arf exchange factors with different sensitivities to brefeldin A control the formation of these different organelles.

Interestingly, Arno, which has a Sec7 domain with a structure very similar to that of Gea2 (Goldberg, 1998), is much less sensitive to brefeldin A, indicating an exquisite specificity. Furthermore, the characterization of the mutations in brefeldin A–resistant mutants of Gea1 defines the site of brefeldin A action on the "Sec7 domain." Four residues appear to be critical for brefeldin A sensitivity: when these positions of Arno or Cytohesin (resistant) are changed to the side chains of Gea1 or p200 (sensitive), Arno or Cytohesin become much more sensitive to brefeldin A (Peyroche et al., 1999; Sata et al., 1999). Thus, the precise site where brefeldin A binds might be known soon, and the structure of the Arf-GDP:brefeldin A:Sec7 domain complex is most likely accessible to crystallographic analysis. These studies might help to design analogs of brefeldin A that would be active on the Arno or EFA6 families and that might inhibit regulated exocytosis and thereby control inflammation or possibly inhibit the entry of some pathogens.

Trapping Proteins in a "Dead-End" Complex: A New Concept for Drug Discovery?

The unexpected discovery that brefeldin A inhibits Arf1 activation by trapping the exchange reaction in a dead-end complex may have profound implications on the development of drugs targeting other exchange factors for small G proteins, and possibly in different biological systems as well. The more obvious approach of searching for compounds that inhibit protein:protein interactions competitively has intrinsic problems. A small molecule is unlikely to bind to a target protein with the same affinity and specificity as its protein partner and is therefore unlikely to be an effective competitor. An inhibitor that binds to a specific site on a protein complex in an uncompetitive manner might be more efficient. Also, it seems that brefeldin A takes advantage of the rearrangements that occur in the Arf-GDP:Sec7 domain complex while GDP is released. The concentration required for a drug to intercalate during this conformational rearrangement might be lower than the concentration required to saturate a protein target with a competitive inhibitor.

Might this mechanism be useful for other targets of drug discovery? Brefeldin A–sensitive Arf1 exchange factors themselves may not be useful targets: they play a major role in constitutive secretion and brefeldin A is therefore rather toxic for most cells. However, inhibitors of exchange factors for other small G proteins, such as Ras or Rac and Rho, might have therapeutic value. For

example, oncogenic transformation by Ras can be blocked by dominant-negative Rac (Qiu et al., 1995). Therefore, a small compound that traps Rac in a complex with the relevant exchange factor could have a similar biological effect. Recent structures of some of these proteins could help guide such drug discovery efforts. The structure of nucleotide-free Ras in complex with the RasGRF domain of Sos shows a large interface between the two proteins where several hydrophobic residues of Sos interact with hydrophobic residues of Ras, mostly in the switch 2 region (Boriack-Sjodin et al., 1998). A hydrophobic molecule might trap Ras-GDP and Sos in a dead-end complex, in the same way that brefeldin A traps the Arf1-GDP:Sec7 domain. A compound that binds to the transient nucleotide-free Ras:Sos complex and impairs GTP entry would presumably be able to block the dissociation of the complex as well, through a distinct mechanism.

The structures of several Dbl homology domains, which promote nucleotide exchange on the Rho family of small G proteins, have also been solved recently, but not in complex with their target. The proposed Rho- or Rac-binding site on Dbl domains appears less hydrophobic than the Ras-binding site in Sos or the Arf-binding site in Arno, suggesting that if a small compound could be found to bind in the interface, it might be less hydrophobic than brefeldin A. However, Soisson et al. (1998) have suggested that the C-terminal part of the Dbl domain of Sos, the region linking it to the PH domain, or perhaps this PH domain itself, could be involved in the interaction with Rac, and there seem to be exposed hydrophobic residues in this region.

This type of mechanism could therefore provide an exquisite selectivity to a compound that might allow discrimination amongst different members of the ever-expanding family of guanine nucleotide exchange factors that are involved in virtually every aspect of cell biology: signal transduction, trafficking, cell motility, nuclear protein export, proliferation, cytoskeletal organization, and so on. Other studies on the mechanism of action of different toxins, such as the cholera toxin (Bornancin and Chabre, 1991), point to the G protein: exchange factor transient complex as a frequent target, although in this case cholera toxin does not impair dissociation but inhibits GTP hydrolysis in a subsequent step. Furthermore, the principle of searching for small molecules that stabilize other types of protein:protein interactions might be worth exploring as a new approach to other potential therapeutic targets. For example, a compound that stabilizes the interaction between oncogenic Ras and GAP might be an effective way of blocking transformation. Moreover, a compound that stabilizes the interaction between MEK and ERKs might block signal transduction by trapping ERKs in the cytoplasm. Considering the number of protein:protein interactions that make attractive targets for intervention, and the comprehensive failure of efforts to find small molecules by conventional inhibitor screens, a new approach may be justified.

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